

## IN THE SPECIFICATION

Please replace the ABSTRACT with the following

The invention relates to polynucleotides which encode a protein kinase A anchor proteins (AKAP) and the use of such polynucleotides to generate AKAP polypeptides or fusion proteins containing AKAP proteins. The present invention also relates to methods of determining the interaction of AKAP proteins with regulatory subunits of protein kinase A and methods for identifying cell-permeable substances.

**Please amend the first complete paragraph at page 22 of the originally-filed specification as follows:**

First of all, the cDNA of a new splicing variant of the protein kinase A anchor protein (AKAP) AKAP18 was identified and isolated (FIG. 1; **SEQ ID NO: 1**), said variant being referred to as AKAP18 $\delta$ . The AKAP18 $\delta$  cDNA was cloned into the commercially available vector PECFP (BD Biosciences, Clontech Heidelberg). The cDNA of the regulatory subunit RII $\alpha$  of human protein kinase A, supplied by Prof. Dr. K. Tasken (University of Oslo), was cloned into the commercially available vector pEYFP (BD Biosciences, Clontech, Heidelberg). Eukaryotic HEK293 cells (GBF, Brunswick) were co-transfected with the plasmids.

**At page 21 of the originally-filed specification, just before the “Examples” section, please add the following new section:**

### BRIEF DESCRIPTION OF THE DRAWINGS

Various features and attendant advantages of the present invention will be more fully appreciated as the same becomes better understood when considered in conjunction with the accompanying drawings, in which like reference characters designate the same or similar parts throughout the several views, and wherein:

FIG. 1 shows the polypeptide (SEQ ID NO: 2) and polynucleotide (SEQ ID NO: 1) sequence of AKAP18 $\delta$ .

FIG. 2 demonstrates co-expression of AKAP18 $\delta$ -CFP and RII $\alpha$ -YFP proteins in HEK293 cells. Expression of RII $\alpha$ -YFP was detected by excitation at 488 nm and measurement of the emitted fluorescence at 535/26 nm (FIG. 2A), and that of AKAP18 $\delta$ -CFP by excitation of fluorescence at

425 nm and measurement of the emitted fluorescence at 480/30 nm (FIG. 2B). FIG. 2C shows a similar distribution of the illustrated YFP emission as in FIG. 2A. Consequently, an energy transfer from CFP to YFP has occurred. FIG. 1D shows a color-coded illustration of the calculated 535/480 ratio of approximately 1.2-1.5 of FRET signals in HEK293 cells.

FIG. 3 is a schematic diagram of fluorescence resonance energy transfer (FRET) technology which is used in the determination of interaction of the expressed fusion proteins AKAP188-CFP and RII $\alpha$ -YFP in a cell.

FIG. 4 shows the specificity of the measured FRET signals, as examined using acceptor bleaching protocol. FIG. 4A describes the kinetics of the fluorescence emitted by YFP and CFP, illustrating the ratio  $F/F_{\max}$  as a function of time (time in s).  $F_{\max}$  corresponds to maximum emission of YFP and CFP, respectively. FIG. 4B shows a regression analysis of  $\Delta F^{\text{CFP}}(\%)$  vs.  $\Delta F^{\text{YFP}}(\%)$ .

FIG. 5A is a schematic diagram showing the inhibition of interaction of AKAP188-CFP and RII $\alpha$ -YFP by peptide S-Ht31. FIG. 5B is a schematic diagram showing the effect of such inhibitory peptides on FRET signals.

FIG. 6A shows the color-coded FRET signal (535/480 ratio) from AKAP188 to RII $\alpha$  in two HEK293 cells. FIG. 6B shows cells incubated with ineffective control peptide S-Ht31-P which has no influence on the AKAP-RII interaction. FIG. 6C shows the summarized data obtained from FIGS. 6A and B, illustrating the change of the FRET signal (in %) as a function of time. The graphic diagram shows the FRET signal change observed on the cells in the presence of S-Ht31 or S-Ht31-P.

FIG. 7 shows that AQP2, PKA and AKAP188 and/or a 55 kDa AKAP are present on the same intracellular vesicles.

FIG. 8 shows that AKAP188 plays a role in the signal cascade resulting in translocation of AQP2 into the apical plasma membrane of renal chief cells. FIG. 8A: CD8 cells were cotransfected with plasmids encoding RII $\alpha$ -YFP and AKAP188-CFP. The FRET was measured prior to and after stimulation of the cells with forskolin (100  $\mu\text{M}$ ). Illustrated are two representative cells, each one co-expressing two fusion proteins. The FRET was measured prior to forskolin administration (0 s) and 95 and 600 s later on (95 s and 600 s). The FRET signal (535/480 nm ratio) is coded in pseudo-

colors. B: Quantitative analysis of the effect of forskolin on the FRET signal (n=6 cells). Scale 20  $\mu$ m.

FIG. 9 is an illustration from Berridge et al. "Calcium signaling: Dynamics, homeostasis and remodelling." *Nature Rev. Mol Cell. Biol.*, 4, 517-529, 2003. The illustration outlines the role of  $Ca^{2+}$  in intracellular signaling and in the remodeling of myocardial cells.

FIG. 10 shows co-localization of AKAP188 with ryanodin R2 receptor 2 (RyR2), SERCA2a, phospholamban (PLN), regulatory RII $\alpha$  and RII $\beta$  subunits of PKA in rat heart cells, as detected via immunofluorescence microscopy.

**Please amend the paragraph bridging pages 23 and 24 of the originally-filed specification as follows:**

The coding region of the AKAP188 cDNA identified by us (FIG. 1; **SEQ ID NO: 1** sequence AKAP188) was amplified using polymerase chain reaction (PCR). To this end, forward primer (position in AKAP188: bp 57-76) having the sequence 5' CTC GAG CTC AAG CTT CGA ATT CTG ATG GAG CGC CCC GCC GCG GG 3' (SEQ ID NO: 3) and reverse primer (position in AKAP188: bp 1095-1118) having the sequence 5' GGC GAC CGG TGG ATC CCG GGC CCG GTT GTT ATC ACT GCC ATC GCC 3' (SEQ ID NO: 4), which bear an EcoRI and a BamHI restriction site, respectively, were employed. The Advantage cDNA Polymerase Mix was used as polymerase according to the manufacturer's instructions. The required 10.times. PCR buffer was supplied together the Advantage cDNA Polymerase Mix. The nucleotides dATP, dCTP, dGTP and dTTP were pipetted into the PCR batch as a dNTP mix (reaction batch see below).